THE EFFECTS OF ULTRA-VIOLET RADIATION OF λ2537A UPON CLEAVAGE OF SEA URCHIN EGGS[1]

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The effect of ultra-violet radiation upon the division rate of various cells has been the subject of a number of investigations, but the results are not in complete agreement, some investigators claiming acceleration (e.g., Coblentz and Fulton, 1924; Hinrichs, 1928; Alpatov and Nastjukova, 1933), others retardation (*e.g.*, Hertel, 1905; Gates, 1929; Oster, 1934; Chase, 1937), still others acceleration or retardation depending upon the wave-length and dosage (*e.g.*, Bovie and Hughes, 1918; Hughes and Bovie, 1918; Hutchinson and Ashton, 1929).

The disagreement may be more apparent than real, the results depending upon the wave-length of the ultra-violet, the dosage and the organism used. Quantitative data are needed to throw further light upon the problem. The following paper is an attempt to gather such data on the effects of one wave-length, 2537A, upon cleavage of sea urchin eggs, this material being chosen because the self-contained food supply greatly simplifies control of the environment. Work is planned at each of the other wave-lengths of ultra-violet light represented in the spectrum of the quartz mercury arc.

Materials and Methods

The sea urchins (*Strongylocentrotus purpuratus* Stimpson) were collected during the winter breeding seasons of 1935 and 1936 at Moss Beach and Pacific Grove, California. Eggs were obtained after natural spawning or by excision of the ovaries and from 25 to 100 eggs were placed in each 1″ watchglass containing 3/4 cc. sea water. The sperm suspension was determined each time by tests, successive dilutions being made until 100 per cent fertilization was achieved without overinsemination, and since almost all eggs cleaved normally, polyspermy was probably rare. Only eggs in which practically 100 per cent showed fertilization membranes within two minutes after insemination were used in this research.

In the case of eggs kept at 14–16° C., the first cleavage occurred in about two hours following insemination, the second after another

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hour, and the successive cleavages to the 64-celled stage at about hourly intervals. Within 20 hours after insemination the actively swimming blastulæ had been formed and in about another 24 hours gastrulation was complete, having begun some 12 hours earlier. Plutei formed within 4 days after insemination although the arms did not begin to elongate for another 24 hours. Since controls showed normal development to the pluteus, the conditions were considered satisfactory for the investigation.

For the first series of studies a mercury-argon discharge tube which emits about 88 per cent of the total output in the visible and ultra-violet at λ2537A (Coblentz, 1931; Leighton and Leighton, 1935) was employed since its high intensity enables one to give an effective dose of radiation in a short time and renders feasible certain experiments otherwise impossible. The intensity of the radiations, after screening out the infra red rays by a suitable water filter, was shown by thermopile measurements to be relatively constant for the period of investigations.

For experiments where pure light of λ2537A was needed the radiations from a water-cooled quartz mercury arc were passed through a natural quartz monochromator and the light of the desired wavelength was focused on the quartz cell containing the eggs. The apparatus used was in general similar to that previously described (Giese and Leighton, 1935).

The line thermopile (type described by Leighton and Leighton, 1932, p. 1884) used in series with a D'Arsonval H.S. galvanometer, was calibrated against Bureau of Standards Lamps C-211 and C-212. The thermopile factor for λ2537A was calculated to be 24.10 ergs/sec./cm. galvanometric deflection.

Experimental

Irradiation of Eggs Just Before the First Cleavage

In the first series of experiments the eggs which had been inseminated 90 minutes previously were irradiated for 1, 2, 4, 8, 16, 32, and 64 seconds at a distance of 31 mm. from the center of the mercury argon tube, and for 1, 4 and 16 seconds at a distance of 248 mm. from the center of the tube (to give exposures approximately equivalent to 1/64, 1/16, 1/4 second). In one series exposures approximately equivalent to 1/8 and 1/32 second were also given. Examinations were made at intervals of a half hour or an hour, depending upon circumstances, and the stages in development recorded. A typical set of data from a series of 3 experiments is plotted in Fig. 1.

It is clear from Fig. 1 that delay in cleavage is proportional to dosage and that even 1 second irradiation is sufficient to delay development for a long time, 1/4 second for a slight period of time. However, the protoplasm of the egg hastily repairs injury from lesser dosages, for example, the rate of cleavage of eggs irradiated 1/8, 1/16, 1/32 and 1/64 second was at no time lower than that of controls. But in no case was an increased rate of cleavage observed.

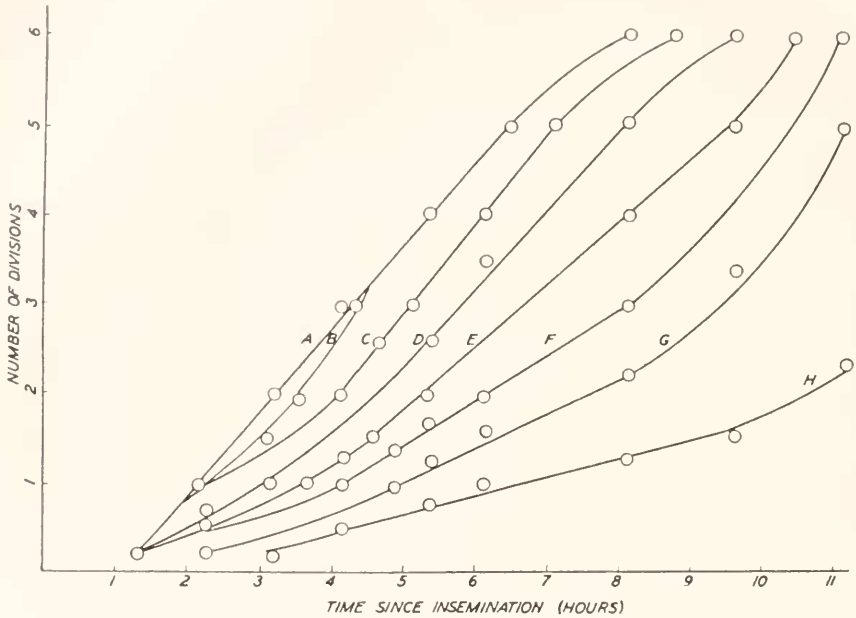

FIG. 1. Retardation of cleavage by radiation.

A. Control.
B. One-fourth of a second dosage.
C. One second dosage.
D. Two seconds dosage.
E. Four seconds dosage.
F. Eight seconds dosage.
G. Sixteen seconds dosage.
H. Thirty-two seconds dosage.

While many of the eggs irradiated for 4 or more seconds developed normally later, in most cases a retardation was observable even at later stages in development. Thus in Table I it will be noted that while 22 hours after insemination normal free-swimming blastulæ were formed from all eggs except those irradiated 4 seconds or more, those developed from eggs irradiated 4 and 8 seconds were still within

the fertilization membranes. Even 32 hours after insemination gastrulation in these eggs was just beginning when the controls and those given smaller doses had completed invagination. Ultimately all the above eggs, even those irradiated as long as 8 seconds, gave rise to gastrulæ normal to all appearances.

TABLE I

Later development of eggs irradiated ninety minutes after insemination at the dosages indicated

Dosage in seconds	22 hours after insemination	32 hours after insemination	46 hours after insemination
1/64, 1/32, 1/16, 1/8, 1/4 and control	Normal free-swimming blastulæ	Normal gastrulæ	Beginning of gut differentiation
1	Normal free-swimming blastulæ	Normal gastrulæ	Gut not yet differentiated
2	Normal free-swimming blastulæ	Normal gastrulæ	Gut not yet differentiated
4	Normal blastulæ but still within membranes	Blastulæ with beginning of invagination	Early gastrulæ
8	Normal blastulæ but still within membranes	Blastulæ with beginning of invagination	Early gastrulæ
16	Mostly abnormal motile balls. Some non-motile blastulæ.	Mostly abnormal. Show delayed gastrulation.	As before
32	Coagulated cells	—	—
64	Coagulated cells	—	—

In some series (5 trials) of eggs irradiated 16 seconds a fair proportion of the eggs formed blastulæ and gastrulæ; in other series of eggs so irradiated cleavage resulted in a mass of motile cells which persisted without differentiation for as long as observations were made. After dosages of 32 seconds eggs developed into masses of undifferentiated cells which never became motile and early appeared opaque. Dosages of 64 seconds apparently inhibited even one division, though in some cases cleavage did occur; more often the attempt at cleavage was abortive, the apparent blastomeres failed to separate, often fused and then cytolized.

Differential Susceptibility of Eggs at Different Stages

It would be of interest to compare the susceptibility of unfertilized eggs, eggs just inseminated, eggs well after insemination, and eggs in the first cleavage. First of all, however, it was necessary to determine whether the unfertilized egg would become activated with the dosages used. Three series of 3 trials each were therefore made with 1/64, 1/16, 1/4, 1, 16, 64, and 256 seconds of irradiation. In no case was a

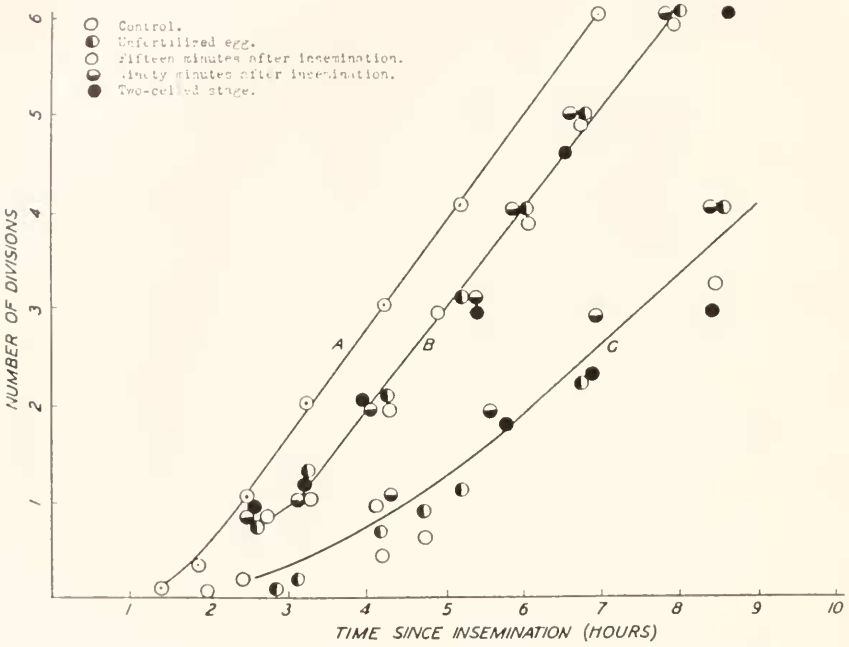

FIG. 2. Differential susceptibility to radiation of eggs at different stages of development.

A. Control.
B. One second dosage.
C. Sixteen seconds dosage.

normal fertilization membrane formed without insemination, nor was division apparent. The dosages used do not, then, activate the eggs to artificial parthenogenesis, but experiments showed that when the dosage was not too great, eggs so irradiated when inseminated would develop normally.

Three series of experiments (3 each) were now performed on eggs in the following stages: (1) unfertilized, (2) 15 minutes after insemina-

tion, (3) 90 minutes after insemination and (4) the two-celled stage with dosages of 1, 4, 16, and 64 seconds of irradiation. The results, excepting those for eggs irradiated 4 and 64 seconds, are given in Fig. 2. It is readily observed that there is no very great difference of susceptibility of these stages, one second of irradiation reducing the cleavage rate about equally in all cases (4 seconds reducing the rate still more, but omitted from the graph to avoid confusion), 16 seconds much more. From Fig. 2 it may be observed that there is a latent period before the effects appear. This is particularly so for all stages irradiated for one second and for the 2-celled stage irradiated at all dosages. The eggs irradiated 64 seconds gave but few abortive cleavages when irradiated before insemination or 15 minutes after insemination. When irradiated for 64 seconds, 90 minutes after insemination, most of the eggs passed into the 2-celled stage, and then cleaved abortively. Apparently at the time of irradiation the mechanism of cell division was already in full swing and could not be stopped. Of eggs irradiated for 64 seconds just after the first cleavage only a small proportion continued to divide and these only for a short time afterwards.

As in the previous experiments retardation was not only obvious during early cleavage but also at later stages, for when controls had developed into free-swimming blastulæ, some of the irradiated eggs had developed into non-motile blastulæ, and when controls had gastrulated, some of those irradiated had only begun to gastrulate. The data for later development are given in full in Table II.

Three sets of eggs in the 2-celled stages were irradiated with dosages equivalent to 1/4, 1/16, and 1/64 seconds, but in *no* case was the cleavage rate greater than that of the controls—in fact, there was a slight delay when the dosage of 1/4 second was given.

Effect upon the Medium

When the mercury-argon discharge lamp is in operation, ozone in readily detectable quantities is produced by the action of the short ultra-violet (1849A) on the oxygen of the air. To determine whether this was dissolving in the medium and causing retarded development in the experiments reported above, sea water was irradiated for 64 and 256 seconds, then eggs were added and the development compared with the controls (5 experiments, 256 seconds; 10 experiments, 64 seconds). Unfertilized eggs, eggs 15 and 90 minutes after insemination and 2-celled stages were used. In all cases there were no signs of retardation, cleavage in all cases being comparable to the controls. The retarded cleavage of eggs irradiated with the mercury-argon tube

is apparently due to the absorption of the ultra-violet radiation by the eggs and not to an effect upon the medium, and it is probably due practically entirely to radiation of λ2537A, since the only lines of

TABLE II

Later development of eggs irradiated at various stages in development

Dosage in seconds	Irradiated at fol. stage in development	21 hours after insemination	Percentage of blastulæ abnormal	27 hours after insemination	45 hours after insemination	No. of eggs used
1	Unfertilized egg	10% non-motile blastulæ	0.0	All motile	Normal gastrulæ	155
	15 min. after insemination	4% non-motile blastulæ	0.0	All motile	Normal gastrulæ	271
	90 min. after insemination	5% non-motile blastulæ	0.5	All motile	Normal gastrulæ	198
	2-celled stage	8% non-motile blastulæ	1.0	All motile	Normal gastrulæ, smaller than controls	188
4	Unfertilized egg	10% non-motile	0.9	All motile	Normal gastrulæ, smaller	346
	15 min. after insemination	77% non-motile	0.0	All motile	Normal gastrulæ	121
	90 min. after insemination	85% non-motile	11.6	All motile	Normal gastrulæ smaller than controls	104
	2-celled stage	99% non-motile	7.6	All motile	Normal gastrulæ, few incompletely invaginated	144
16	Unfertilized egg	All non-motile	0.0	Almost all non-motile	Some abnormal; most smaller than controls	250
	15 min. after insemination	All non-motile	13.7	20% motile	16% normal gastrulæ. Others abnormal morulæ	257
	90 min. after insemination	All non-motile	24.0	76% motile	87% gastrulate, small, somewhat abnormal	175
	2-celled stage	All non-motile	30.8	5% motile	5% normal gastrulæ, others abnormal morulæ	94
0	Controls	Motile blastulæ	0.4	Motile blastulæ	Gastrulæ	232

significant intensity in the mercury-argon tube spectrum are 3130, 3660, and several visible lines (4050, 4358, 5461, 5790A), all of which are relatively inert.

Experiments with Pure Light of λ2537A

To be certain that monochromatic light of λ2537A is the effective agent, a number of experiments were tried with pure light of this wavelength obtained from the monochromator already described. The unfertilized eggs were placed in a quartz cell and irradiated in the manner described in the previous paper (Giese and Leighton, 1935) usually for periods of 1, 4, 16, 64, 256 and 1,024 seconds. They were then trans-

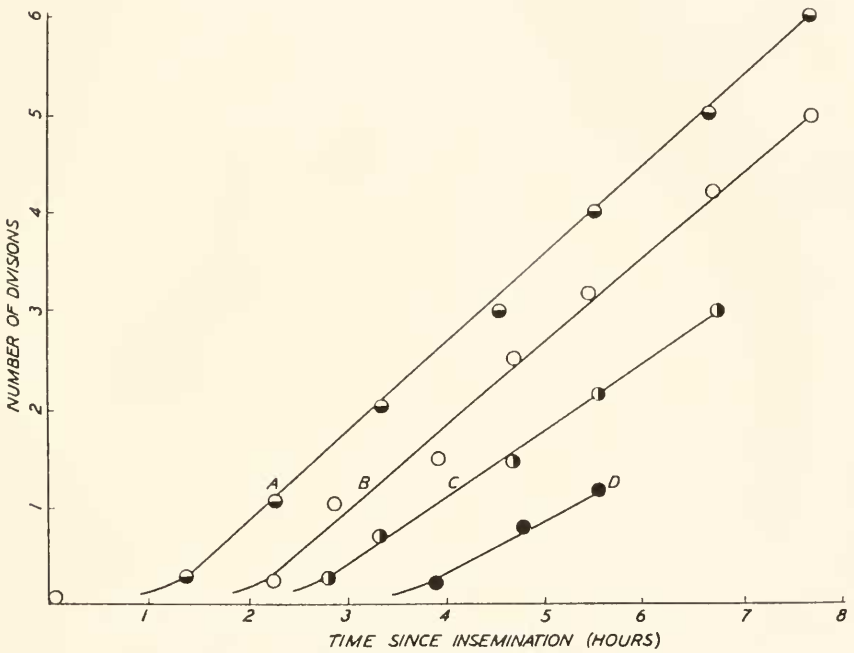

FIG. 3. Retardation of cleavage, λ 2537A; intensity, 9.74 ergs/mm.²/sec.
A. Control.
B. Sixty-four seconds exposure, extinction per egg: 2.91 ergs or 3.74×10^{11} quanta.
C. Two hundred and fifty-six seconds exposure, extinction per egg: 11.67 ergs or 1.50×10^{12} quanta.
D. One thousand and twenty-four seconds exposure, extinction per egg: 46.69 ergs or 6.00×10^{12} quanta.

ferred to watchglasses, inseminated and observed at hourly intervals until they had reached the 64-celled stage and then at 12-hour intervals until the gastrula stage. Seven series of experiments, the last three covering only the longer exposures, gave similar results and one series is plotted in Fig. 3.

It will be noted that retardation is evident when the dosage is

large enough. Smaller doses produced no noticeable retardation or acceleration. The results are in general similar to those already obtained with the mercury-argon discharge tube.

Extinction Measurements

It would be interesting to know the amount of energy which must be absorbed to produce the above effects. By interposing a cell first empty then full of eggs between the thermopile and the light source one can determine the fraction of the light incident upon the cell which is transmitted, I/I_0, where I is the intensity of the transmitted light and I_0 the intensity of the incident light. The fraction of the light extinguished, i.e., lost on passage through the cell can then be determined by subtracting the fraction transmitted from unity, $1 - I/I_0$. By determining the fraction of the area of the cell occupied by the eggs, A_e, one can determine the fraction of the light incident upon the eggs which is extinguished, $1 - I/I_0 \times 1/A_e$.

TABLE III

Data on extinction of light by sea urchin eggs

Exp.	No. eggs	A_e, fraction of area of cell bottom covered by eggs	$1 - I/I_0$ for λ's below:					$1 - I/I_0 \times \frac{1}{A_e}$ for λ's below:				
			2537A	4350A	5461A	5844A	6904A	2537A	4350A	5461A	5844A	6904A
1	3320	0.49	0.51	—	—	—	0.13	1.04	—	—	—	0.26
2	4431	0.65	0.63	0.38	0.27	0.23	0.14	0.97	0.58	0.42	0.35	0.22
3	3932	0.58	0.66	—	0.34	0.26	0.14	1.14	—	0.58	0.45	0.24
4	5964	0.88	—	—	—	0.38	0.25	—	—	—	0.43	0.28

A_e can be determined from the number of eggs, which can be counted, and the area of the egg effective in extinguishing light, which is approximately the area of a circle of the diameter of the egg. The average diameter of the egg used was the mean of the diameters of 50 eggs taken at random, $77.2 \pm 2.9\ \mu$ (two diameters were measured since almost one-half of the eggs were slightly ellipsoidal). A circle of this diameter has an area of $4{,}681\ \mu^2$. About 6,765 eggs would be necessary to completely cover the bottom of the cell used (area, $31.67 \times 10^6\ \mu^2$) with a layer one egg diameter thick. A_e is the number of eggs counted in a given experiment divided by 6,765.

The transmission of light of various wave-lengths by eggs was determined, the method used being similar in practically all respects to that described for *Paramecium* (Giese and Leighton, 1935), and the number of eggs was counted in each experiment. The experimental and derived data are recorded in Table III.

It is clear that practically all the light of λ2537A incident upon the eggs is extinguished, while at the longer wave-lengths a much smaller proportion is so lost. Part of the light extinguished is truly absorbed, part is lost by scattering from the surface of the egg and from the surfaces of small particles within the egg. Unfortunately it is very difficult to measure the scattering and it is impossible to obtain an approximation of the scattering as was done with *Paramecium* since unlike the latter the eggs absorb in the visible part of the spectrum as is quite obvious from the data in Table III. It is probable that as for *Paramecium* a considerable proportion of the light extinguished is actually scattered, possibly as much as 50 per cent of the total. However, the extinction measurements at least give the order of magnitude of the energy involved. The extinction by the eggs for the various experiments in Fig. 3 has been determined and the data have been added to that figure.

Discussion

The data of Fig. 3 are interesting because they give an idea of the number of quanta which must be absorbed to produce an effect. Thus eggs extinguishing between 1.57×10^9 and 2.54×10^{10} quanta, and probably absorbing about half this quantity, were not visibly affected and cleaved comparably to controls, only after a dosage of about 3.74×10^{11} quanta per egg was the rate of cleavage definitely retarded. Only doses short of those producing cytolysis stop cleavage for eggs extinguishing 1.24×10^{13} quanta went on developing as far as the 8-celled stage in many cases. Were one to assume that the average molecule in the egg protoplasm had a mass of the order of magnitude of the mass of the egg-albumin molecule, one would find the egg to possess some 2.5×10^{12} molecules. The actual number is probably much larger, but the figure indicates that a fair proportion of the molecules are affected or that certain molecules have absorbed many quanta before an effect is evident. The data are also indicative of the high power of recovery from injury possessed by the egg protoplasm.

From the data presented one may conclude that for the wave-length and the dosage series used, which covers the range usually employed in similar experiments, there is no evidence of acceleration of cell division. Following large doses of radiation there is retardation; following smaller doses the rate of cleavage is not noticeably different from controls.

These results do not, however, exclude the possibility of a stimulative effect of doses of ultra-violet light much weaker than here

employed. The radiations claimed by the Gurwitsch school of mitogenetic rays to be short ultra-violet and to be effective in increasing mitoses are postulated to be an entirely different order of magnitude from the radiations here used, in fact so weak as to defy physical detection. No attempt is here made to throw light upon this complex problem (see Rahn, 1936).

Summary

1. There is a threshold dosage between 10^{10} and 10^{11} quanta below which radiation of λ2537A produces no observable change in the rate of cleavage. Beyond this threshold the degree of retardation increases with the dose.

2. Many of the retarded eggs develop normally, but are slower in reaching a given stage; others continue developing for only a short time, the degree of differentiation reached being inversely proportional to the dosage.

3. Unfertilized eggs, eggs 15 and 90 minutes after insemination, and eggs in the first cleavage do not exhibit strikingly different susceptibilities to the rays, although the later stages appear to be somewhat more susceptible.

4. The quantity of radiant energy which the eggs can absorb before being affected is quite large, as indicated by the extinction measurements reported, and serves as a rough measure of the power of repair of the egg protoplasm.

5. A series of dosages from a dose which cytolyzes to one which has no retarding effect upon cleavage with light of λ2537A failed to induce artificial parthenogenesis.

6. No evidences were obtained over the dosage series investigated for acceleration of the rate of cleavage.

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